# A NEW METHOD FOR THE MEASUREMENT OF SALIVATION IN SMALL RODENTS*

by

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# A New Method for the Measurement of Salivation in Small Rodents*

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Mr. Chairman, members of the Society:

Studies on the salivary glands and their secretions are important with respect to nutrition, digestion, and dentistry, as well as with regard to excretion from circulation, and the neural and endocrinological regulation of glandular function.

The literature does provide a variety of methods to measure salivation. These have most frequently been worked out for the larger farm animals, as well as larger laboratory animals, such as dogs, cats, rabbits and rats. There are few methods available for smaller experimental animals, in particular, for hamsters or mice. This is due primarily to difficulties in the collection of saliva from these small species. Quite recently, however, some methods have been proposed for the measurement of salivary performance in mice. These procedures require anesthetized animals, and stimulation of salivation by drugs is essential in order to obtain information on the inhibition of salivation by various factors. Moreover, their monitoring system is not sensitive enough to quantitate salivation in the pseudo-physiological range (Richter; Lavy and Mulder; Aust).

To the best of our knowledge, the literature does not provide a simple method for the measurement of salivation in unanesthetized small rodents under pseudo-physiological conditions.

It is the purpose of this paper to present a nontraumatic method for the quantative monitoring of salivation in minimally restraint unanesthetized mice. Narrow cotton fabric strips pretreated with Cresol Red indicator are inserted into the mouth by means of an oral wire bit to absorb saliva. The length of the diffused colored band, visible after a given time interval, is measured in mm, and indicates the amount of saliva excreted. Immobilization of the mice for measuring salivation is achieved by fitting permanent "halters" around the neck of the animals and inserting the halter into a "stancion" during measurements. Such an arrangement permits the simultaneous salivation study of a large "herd" of mice.

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Slide I shows a closeup of a mouse in the stancion. The mouse wears a felt collar permanently around its neck. A number is engraved into the collar for identification of the mouse. During the experiment, the collar is fixed into this plexiglass holder. A wire "bit" is placed into the mouse's mouth. A narrow cotton strip is attached to the "bit." The strip turns from yellow to purple upon exposure with saliva.

Slide II shows, that the described setup does permit the simultaneous study of large numbers of mice. It demonstrates further that application of treatments can be done in a very systematic fashion. On this picture you see the salivary performance by various strains of mice (on which we will report later).

In order to standardize the method, spontaneous salivation was measured under a variety of experimental conditions over a ten-minute period. Salivation was found to be significantly affected, among other factors, by the number of consecutive days during which each mouse was subjected to measurement, the frequency of measurements during each day, and variations from one animal to another.

To further standardize the methodology, the method was applied to examine the presence or absence of any systematic differences among both sexes of various strains of mice. Twenty animals (half males, half females) of each of four inbred strains of mice, namely AKR/J, DBA/2J, C3HeB/FeJ and C57BL/6J from the Jackson Laboratories in Bar Harbor were studied. Salivation was measured over a four-day period, six times daily at hourly intervals.

Slide III presents the design of this experiment, involving a total of 80 mice and 1,920 individual salivation determinations.

The mice, all ten weeks old, were housed five to a cage for a period of two weeks prior to the actual start of the salivation experiment. During the first week, all mice were fitted with collars and numbered. The mice had no prior exposure to the salivation technique. The data were systematically recorded. They were analyzed statistically, primarily by means of the analysis of variance, using the 370 Wang computer system.

We will limit ourselves to statistically significant results only, and exclude nonsignificant data or trends from further considerations.

Slide IV shows graphically the salivating performance for the four different strains of mice on four consecutive days. Significant differences among these strains were found, except between DBA/2J and the C57BL/6J. Thus, the AKR/J exhibits the lower salivation, salivary performance of the C3HeB/FeJ is intermediate, and DBA/2J and C57BL/6J are both high salivators.

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Slide V shows the salivary performances of mice in each sex of each strain. Statistical analysis of the data proved that male DBA/2J mice produced less saliva than the females of this strain. This sex difference was reversed in the C3HeB/FeJ, where the males produced more saliva than the females. No sex differences were noted in the low-salivating AKR/J and the high-salivating C57BL/6J mice.

Note further that salivation is significantly lower during the first day than in the three following days. This holds true for each sex of each of the four strains. The differences in salivation during the last three days are not significant, except for the AKR/J females, where salivation is increased progressively during the four-day period of experimentation.

We have done further statistical analysis on these data.

Slide VI provides a summary of the various factors that contribute to salivation differences in each sex and strain during this experiment.

The results show that in nearly all cases, more than 95 per cent of the variability in salivation can be accounted for by controlled factors, thus leaving only a 5 per cent random variability. This indicates that the experimental conditions within the inbred strains of mice are very well defined. Approximately 80 per cent of the variability in salivation can be attributed to individuality among the animals and to days of experimentation. The rank order of the measurement during the day, and the "Day X measurement" interaction, are only minor contributing factors to variability and were found to be nonsignificant. This means, in practice, that the spontaneous salivation during any one day is rather stable, when salivation is measured at hourly intervals.

In future biological assays, we will utilize the contributions to variability by "animals" but will eliminate the contribution to variability by "days" through omission of first day salivation data from mice not previously used.

As an example in pharmacology, a pilot study was performed examining the effect of methacholine on salivary performance of random-bred male Ha/ICR Millerton Swiss mice, a noninbred strain which exhibits much more variability in salivating performance than the inbred strains of mice, presented above).

Slide VII shows the results obtained. Note the large stimulation of salivation when the mice were injected subcutaneously with methacholine, immediately prior to the third salivation.

In summary, a new method has been described for the measurement of salivation in small rodents. The method employs minimally restraint, unanesthetized mice. The method is suitable for physiological and pharmacological studies, and data can be obtained simply and in adequate numbers to permit statistical analysis of results.

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## EXPERIMENTAL DESIGN (STRAIN STUDY)

| STRAIN | SEX | NO. ANIMALS | DAYS | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 |
| AKR/J | M | 10 | | | | |
| | F | 10 | | | | |
| DBA/2J | M | 10 | | | | |
| | F | 10 | | | | |
| C3HeB/FeJ | M | 10 | | | | |
| | F | 10 | | | | |
| C57BL/6J | M | 10 | | | | |
| | F | 10 | | | | |

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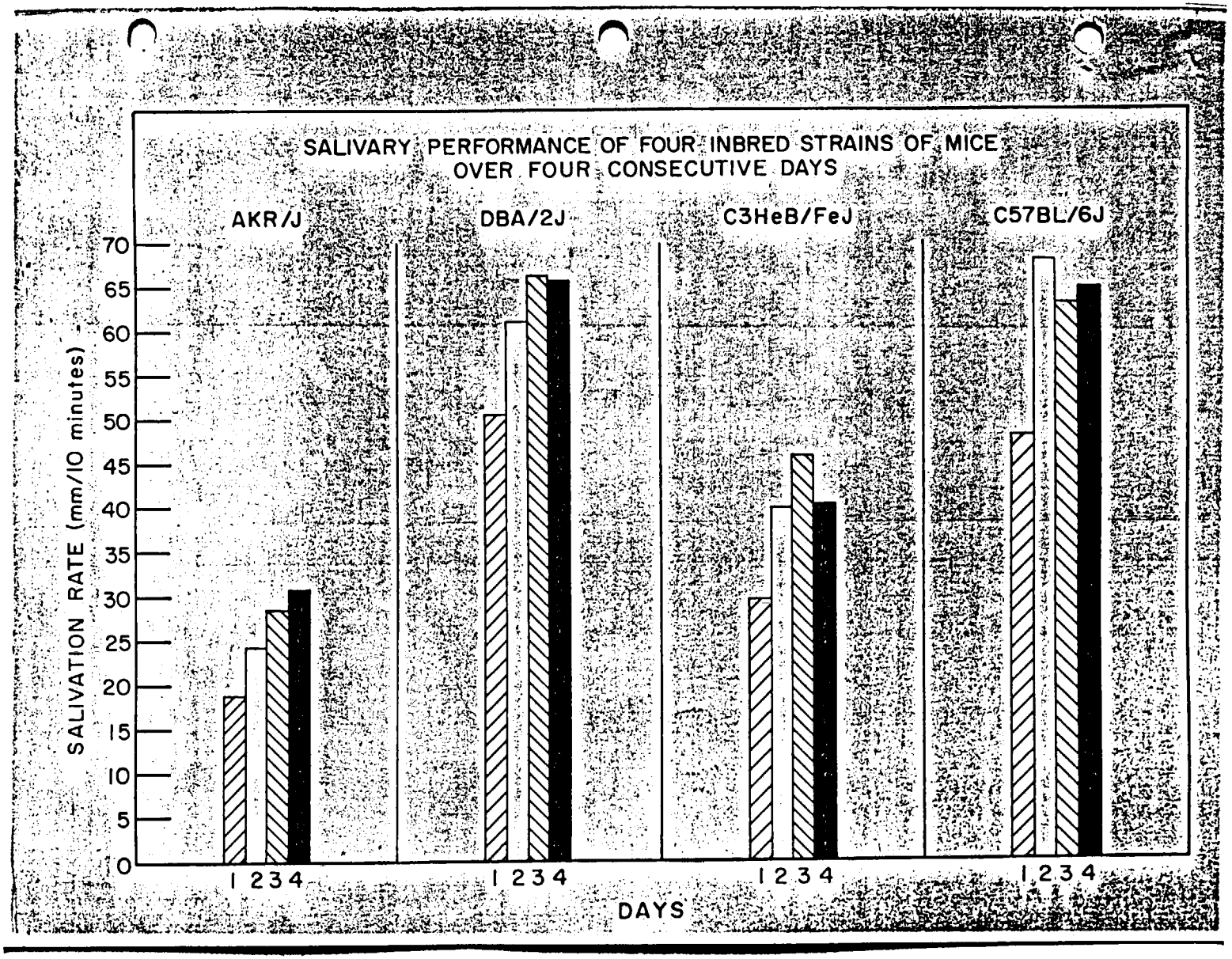


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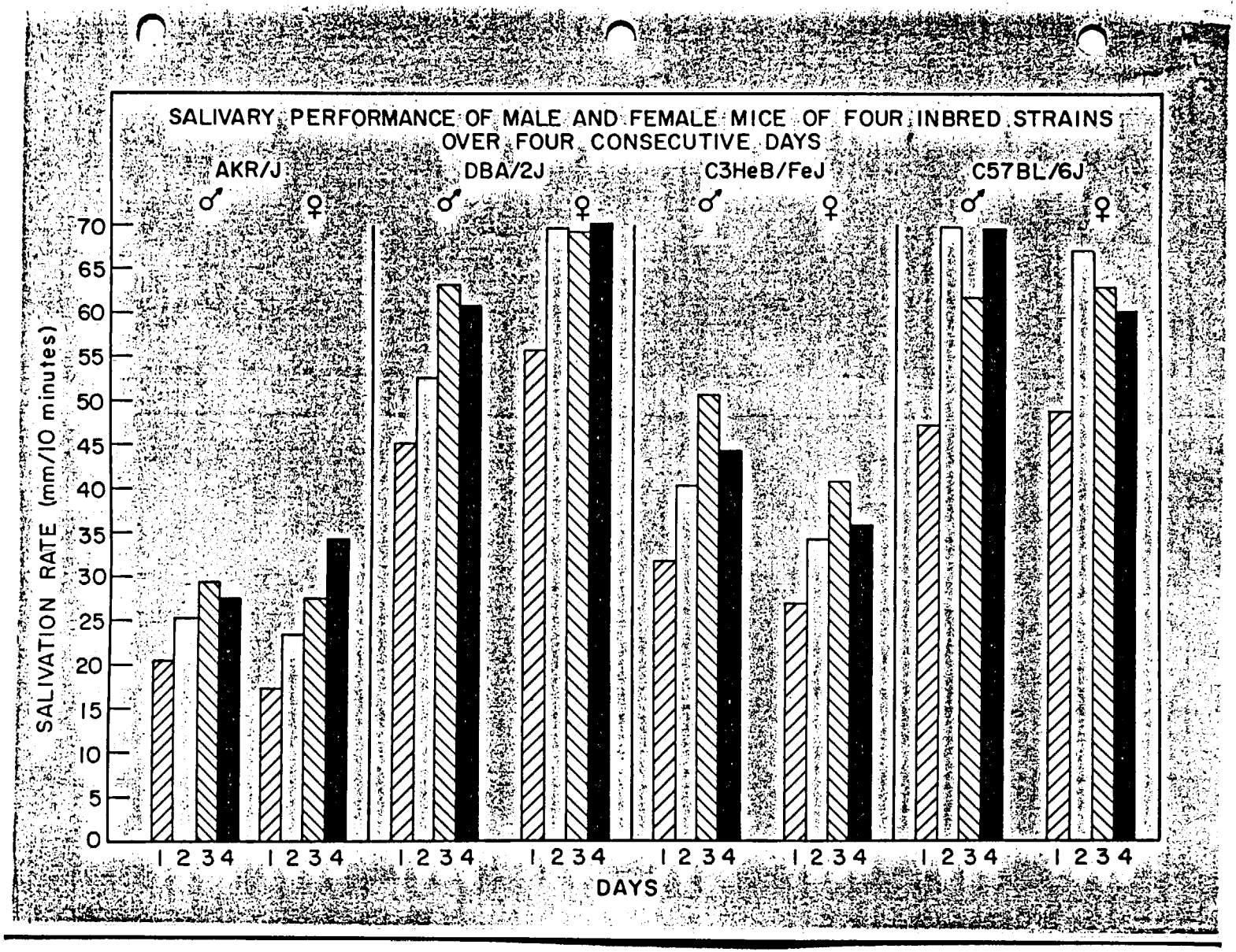


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Summary of Factors Contributing to Total Variance in Salivary Performance in Each Sex of Four Inbred Strains of Mice (Expressed in Percentages)

| Strain | AKR/J (I) | | DBA/2J (II) | | C3HeB/FeJ (III) | | C57BL/6J (IV) | |
|---|---|---|---|---|---|---|---|---|
| Sex | M | F | M | F | M | F | M | F |
| Animals | 59** | 30** | 42** | 58** | 40** | 33** | 33** | 34** |
| Times | 6* | 9** | 8** | 6 | 8** | 9 | 11** | 12** |
| Days (D) | 21* | 56** | 39** | 27** | 38** | 46** | 40** | 31** |
| Measurements (M) | 7 | 1 | 5 | 3 | 7 | 2 | 7 | 9** |
| D x M | 3 | 2 | 3 | 3 | 3 | 4 | 6 | 10** |
| Error | 4 | 2 | 4 | 4 | 4 | 7 | 3 | 4 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

** Significant at the 1% level of probability.
* Significant at the 5% level of probability.

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THE EFFECT OF METHACHOLINE Cl ADMINISTERED SUBCUTANEOUSLY IMMEDIATELY PRIOR TO THE THIRD MEASUREMENT ON SALIVARY PERFORMANCE OF MALE Ha/ICR MILLERTON SWISS MICE

| TREATMENT | MEASUREMENT | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| CONTROL | 183* | 127 | 164 | 159 |
| 0.083 MG | 141 | 188 | 373 | 143 |
| 0.833 MG | 157 | 103 | 812 | 97 |
| 8.333 MG | 154 | 196 | DIED | --- |

* MEAN OF TEN SALIVATION MEASUREMENTS, EXPRESSED IN MM.

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